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Genetic similarities among cocoyam cultivars based on randomly amplified polymorphic DNA (RAPD) analysis

R.J. Schnell[a,*], R. Goenaga[b], C.T. Olano[a]

[a] *National Germplasm Repository, USDA-ARS, 13601 Old Cutler Rd., Miami, FL 33158, USA*
[b] *Tropical Agriculture Research Station, P.O. Box 70, Mayagüez, PR 00681-0070, USA*

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Abstract

Eighteen cultivars of cocoyam (*Xanthosoma* spp.) and two cultivars of taro (*Colocasia esculenta* (L.) Schott) from the USDA/ARS germplasm collection were evaluated for genetic relatedness using RAPD data. Seven random primers generated 40 RAPD loci. Of the 18 cultivars screened, 11 (61%) were identical at all RAPD loci evaluated. A similarity matrix was constructed on the basis of the presence or absence of bands. Among cocoyam cultivars the genetic similarity ranged from 0.86 to 0.97 with a mean of 0.91. Cluster analysis identified two main clusters with some unexpected groupings. These data indicate that very little genetic variation exists within the accessions used in this study and that this *Xanthosoma* spp. collection is of limited value as a genetic resource.

Keywords: *Xanthosoma*; *Colocasia*; Cocoyam; Tanier; Taro; Genetic diversity; RAPD; PCR; Molecular markers

1. Introduction

Cocoyam (*Xanthosoma caracu* Kock and Bouche and *X. violaceum* Schott) is a herbaceous perennial root crop belonging to Araceae family. It is of tropical American origin but many of its species are now widely distributed in Africa,

* Corresponding author.

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Oceania, and Southeast Asia. *Xanthosoma* spp. have a multiplicity of common names including malanga in Cuba, yautía in the Dominican Republic and Puerto Rico, tannia in Trinidad, and cocoyam in Ghana and Nigeria (Morton, 1972). The plant is characterized morphologically by a subterranean stem or corm enclosed by dry, scale like leaves. Secondary corms or cormels arise vegetatively from the main corm. The cormels are used for human consumption while the corms are used for vegetative propagation. Another member of the Araceae, taro (*Colocasia esculenta* (L.) Schott) is cultivated for its corms and is closely related to cocoyam.

Although many developing countries in the tropics depend on cocoyam and taro as a source of carbohydrates, the importance of these genera's adaptability, acceptance, and commercial food value, has received little attention (Goenaga et al., 1991). Studies conducted by Goenaga and Chardón (1993, 1995) demonstrated that cocoyam and upland taro yields can reach 34 000 and 20 000 kg ha^{-1}, respectively, illustrating the commercial potential of these crops.

Accessions of cocoyam in the germplasm collection at the Tropical Agriculture Research Station (TARS), located in Mayagüez, Puerto Rico, are grown in the field and regenerated vegetatively by replanting every 10–12 months. The expense associated with this type of maintenance is only justified when the collection is well characterized and genetically diverse (Jarrett and Florkowski, 1990). The similarity of common names and lack of obvious phenotypic variation among many cocoyam accessions led the authors to suspect a high degree of genetic relatedness. One of the initial steps towards improving management efficiency is the estimation of the genetic diversity within the collection and elimination of duplicate accessions (Greene and Pederson, 1996). Subsequently, efforts can be made to bolster the collection with more morphologically and genetically diverse accessions collected from the crop's center of diversity.

Random amplified polymorphic DNA (RAPD) markers have proven to be a reliable method for fingerprinting accessions in germplasm collections. These markers have also been used to estimate genetic relatedness, they have the advantages of simplicity and the ability to detect relatively small amounts of genetic variation (Skroch and Neinhuis, 1995). RAPD markers have limitations including questionable reproducibility of some bands, a requirement for stringent standardization of reaction conditions, comigration of different amplification products, and dominant inheritance (Bachmann, 1994). Despite these caveats the RAPD method offers the highest potential for generating large numbers of markers with the greatest ease. Schnell et al. (1995) demonstrated that almost all south Florida mango cultivars could be distinguished using just two RAPD primers. The technique has been used successfully with other vegetatively propagated crops including apple (Koller et al., 1993), grapes (Qu et al., 1996), annonas (Ronning et al., 1995) and avocado (Fiedler and Bufler, 1995).

Our objective was to determine the amount of genetic diversity within the selected *Xanthosoma* accessions. To this end we developed a protocol for RAPD

analysis of cocoyam and taro and screened the selected accessions within the germplasm collection using these markers. Herein we report on the usefulness of RAPD markers for fingerprinting accessions and for estimating genetic diversity in cocoyam.

2. Materials and methods

2.1. Plant material

Tissue samples were collected from 14 accessions of *X. caracu*, three accessions of *X. violaceum*, one accession of *Xanthosoma* spp. and two accessions of *Colocasia esculenta*, maintained at TARS in May 1996 (set 1) and again in May 1997 (set 2) (Table 1). Young leaves were collected and shipped chilled to the National Germplasm Repository (NGR) at Miami, FL. Samples were weighed and stored at −80°C.

Table 1
Xanthosoma and *Colocasia* cultivars included in the diversity study and grouped by identical RAPD banding patterns

Group	Cultivar	Interior cormel color	Species	Source
Xanthosoma				
A	Las Mesas	White	*X. caracu*	Puerto Rico
	Blanca	White	*X. caracu*	Puerto Rico
	Blanca Del Pais	White	*X. caracu*	Puerto Rico
	Bisley	White	*X. caracu*	Trinidad
	Charanelle	White	*X. caracu*	Trinidad
	Florida White	White	*X. caracu*	Florida
	Santo Domingo White	White	*X. caracu*	Dominican Republic
	Blanca Espanola	White	*X. caracu*	Puerto Rico
	Inglesa	White	*Xanthosoma* spp.	Puerto Rico
	Aguadillana	White	*X. caracu*	Puerto Rico
	Viequera	White	*X. caracu*	Puerto Rico
B	Vinola	Violet	*X. violaceum*	Puerto Rico
	Morada	Violet	*X. violaceum*	Puerto Rico
C	Santo Domingo Purple	Violet	*X. violaceum*	Dominican Republic
	Alela	White	*X. caracu*	Puerto Rico
	Barbados	White	*X. caracu*	Barbados
	Chowbutton	White	*X. caracu*	Trinidad
	Drearies	White	*X. caracu*	Trinidad
Colocasia (Taro)				
	Lila	Violet	*C. esculenta*	Puerto Rico
	Bun Long	Violet	*C. esculenta*	Hawaii

2.2. DNA extraction and quantification

DNA extraction was performed following the method used by Ronning et al. (1995). One gram frozen leaf samples were ground in a mortar with liquid nitrogen. Ten ml of extraction buffer [100 mM Tris–HCl, pH 8.0; 250 mM NaCl; 25 mM ethylenediaminetetraacetic acid (EDTA); 0.1% (v/v) 2-mercaptoethanol; 100 mM diethyldithiocarbamic acid (DEDTC); 2% (w/v) polyvinylpolypyrrolindone (PVPP)] were added and grinding continued. The tissue homogenate was filtered through four layers of cheesecloth. Sodium dodecyl sulfate (SDS) was added to a final concentration of 1.25%, the mixture was shaken vigorously and incubated at 65°C for 10 min. Potassium acetate was added to a final concentration of 1.2 M, shaken vigorously and incubated at 0°C for 20 min followed by centrifugation at 25 000 *g* for 20 min at 4°C. The supernatant was filtered through two layers of miracloth, combined with 2/3 volume ice cold isopropanol and precipitated at −20°C for 30 min or overnight. DNA was pelleted at 20 000 *g* for 15 min at 4°C. The pellet was dried and resuspended in 400 μl Tris–EDTA (TE) pH 7.4. The samples were treated with ribonuclease A (RNase A, (Promega, Madison, WI)) by adding RNAse A to a final concentration of 50 mg ml^{-1} and incubated at 37°C for 30 min. Organic extractions were performed by adding an equal volume of Phenol : Chloroform : Isoamyl Alcohol, shaking vigorously and centrifuging at 12 000 *g* for 10 min; then the process was repeated with Chloroform : Isoamyl alcohol. DNA was then precipitated by adding NaCl to a final concentration of 0.2 M and two volumes of ice cold 95% Ethanol, incubated at −20°C for 30 min or overnight and centrifuged for 15 min at 12 000 *g*. The pellet was dried and then resuspended in 100 μl TE pH 7.4. DNA concentration was determined by the use of a DynaQuant 200 fluorometer (Hoefer Pharmacia, San Francisco, CA).

2.3. DNA amplification

PCR amplifications were carried out using RAPD 10 mer primers (Operon Technologies, Alameda, CA). The 18 μl PCR reaction mixture contained: 10 mM Tris–HCl, 10 mM KCl pH 8.3, 4.3 mM $MgCl_2$, 0.17 μM each of dATP, dCTP, dGTP and dTTP, 0.5 μm primer, 12 ng genomic DNA, and 1.6 U Amplitaq DNA polymerase, Stoffel fragment (Perkin Elmer, Foster City, CA). Samples were overlaid with mineral oil. Amplification was performed using a Perkin Elmer Cetus Thermal Cycler 480, preheated to 94°C. The reaction mixtures were subjected to 94°C for 2 min, followed by 45 cycles of 94°C for 15 s, 37°C for 30 s, 72°C for 1 min, and then a final 5 min 72°C extension. The RAPD PCR products were electrophoresed in 2% agarose (Sigma, St. Louis, MO) gels with 0.5 X Tris–Borate EDTA Buffer at 5 V cm^{-1}, visualized by ethidium bromide

staining and imaged with a digital camera (UVP, Upland, CA) using Grab-it software (UVP).

2.4. Primer screening

A subset of four cultivars from diverse origins were amplified with 59 RAPD primers. Amplification products were assessed for the number and quality of polymorphic loci. Seven primers that amplified reproducible polymorphic bands were selected for the analysis. DNA was isolated from each sample in sets 1 and 2. All samples were amplified twice to verify patterns and reproducibility. If ambiguous results were obtained from a given amplification then the amplification was repeated. Only reproducible bands in multiple, independent runs from both extractions (sets 1 and 2) and between replicate amplifications within extractions were scored. A RAPD locus as described here consists of a set of comigrating RAPD fragments amplified by the same RAPD primer (Skroch and Neinhuis, 1995). RAPD loci were scored as 0 (band absent) or 1 (band present).

2.5. Data analysis

The gel images were analyzed using ProScore software (DNA ProScan, Nashville, TN) for estimation of the molecular weights of bands. Statistical analysis was carried out using the computer program NTSYS (Exeter Software, Seauket, NY). A pairwise similarity matrix between genotypes was generated using the Dice coefficient (Dice, 1945). The similarity coefficient, S_{ij}, equals $2N_{ij}/(N_i + N_j)$, where N_{ij} is the number of positive bands shared by both individuals i and j. N_i and N_j are the numbers of fragments present in individuals i and j (Dudley, 1994). The SAHN clustering program was then used to group the entries based on similarity coefficients using the unweighted pair-group method using arithmetic average (UPGMA). A phenetic tree was constructed and confidence limits placed on the dendrogram using the bootstrapping program WinBoot with 1000 bootstrap replications (Yap and Nelson, 1996).

3. Results and discussion

Amplification of genomic DNA using the seven primers yielded 40 reproducible RAPD loci for an average of 5.7 bands per primer. Of these, 38 (95%) were polymorphic and two (5%) were monomorphic. Among the cocoyam cultivars only seven bands (17.5%) were polymorphic with the rest of the polymorphism existing between the *Xanthosoma* and *Colocasia* groups (Table 2).

Table 2
Primers and basepair lengths of RAPD generated markers

Primers	Basepair length (bp)
OPA-5	350, 440, 475, 520, 620, 700, 720[b], 770[b], 950, 980, 1070
OPB-4	830[b], 960, 1000
OPB-10	375, 400[b], 470, 490, 530, 630, 670, 870, 1130
OPD-16	400[b], 520[a], 550, 780
OPE-9	530, 900, 1140[b]
OPI-16	340, 565, 580, 610, 800
OPJ-1	450, 530[a], 650, 700, 1230[b]

[a]Monomorphic.
[b]Polymorphic within *Xanthosoma*.

The cocoyam cultivars were placed into groups based on identical RAPD banding patterns. All accessions within a group had identical banding patterns for all 40 RAPD loci and were considered identical for computational purposes (Table 1). Group A consists of 11 (61%) of the cocoyam accessions. Within this group the accession Inglesa is listed as *Xanthosoma* spp., however it is likely, based on its phenotype and RAPD banding patterns that it is *X. caracu*. Group B consists of two accessions, Vinola and Morada both classified as *X. violaceum*. Group C consists of two accessions, Santo Domingo Purple (*X. violaceum*) and Alela (*X. caracu*). This grouping was unexpected as these two accessions are classified as different species that differ in interior cormel color which is a primary identifier for these species. Barbados, Chowbutton, and Drearies each have unique banding patterns. The two *Colocasia* accessions, Lila and Bun Long, were identical.

Banding patterns for representatives of the various groups and individuals are illustrated in Fig. 1, using primers OPA5 and OPB4. Lanes 1, 9, and 17, are a 100 base pair (bp) standard (Gibco BRL, Gaithersberg, MD). Florida White (Group A) is amplified in lanes 2 and 10, Barbados in lanes 3 and 11, Chowbutton in lanes 4 and 12, Morada (Group B) in lanes 5 and 13, Drearies in lanes 6 and 14, Santo Domingo Purple (Group C) in lanes 8 and 15, and Bun Long (*Colocasia* group) in lanes 7 and 16. Differences between the cocoyam and taro accessions are apparent for primer OPA5 band OPA5-1070 and primer OPB4 band OPB4-960 which are present in Bun Long but absent in all *Xanthosoma* accessions. The polymorphic band OPA5-770 is present in Bun Long and in Barbados but absent in all other *Xanthosoma* accessions. Polymorphic band OPA5-720 is present in Morada, Drearies, and Santo Domingo Purple and absent from Florida White, Barbados, Chowbutton and Bun Long. For primer OPB4 differences can be seen for band OPB4-830 which occurs in Florida White, Barbados, Chowbutton and Morada, but is absent in Drearies, and Santo Domingo Purple and Bun Long (Fig. 1).

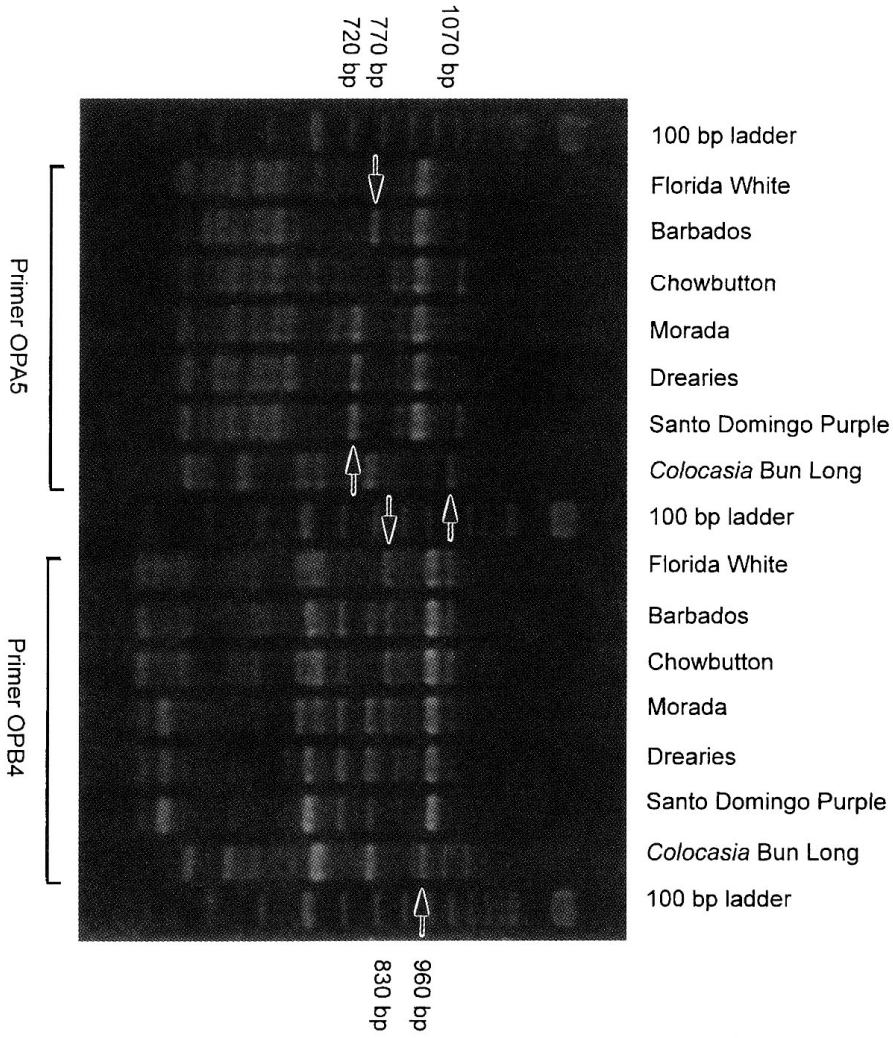

Fig. 1. Amplification products obtained from primers OPA5 and OPB4. The polymorphic markers are indicated by arrrows and include OPA5-1070, OPA5-770, OPA5-720, OPB4-960 and OPB4-830.

The cocoyam cultivars had a high genetic similarity with a range of 0.86–0.97 and an average of 0.91. Accessions in Group A had a high genetic similarity with both Barbados and Chowbutton, 0.97, and also with Group B, 0.92. A high genetic similarity was also found between Drearies and Group C, 0.97. These high numeric similarities suggest a common lineage, although this could not be confirmed from the available passport data. These data indicate that very little genetic variation exists between these groups. The taro accessions had a low genetic similarity with the cocoyams averaging 0.12 (Table 3). Overestimation of genetic similarity using RAPD markers cannot be discounted. However, in this study great care was taken to standardize reaction conditions and score only bands reproducible over the duplicate extractions and replicate amplifications within extractions.

Table 3
Pair-wise similarities based on randomly amplified polymorphic DNA analysis for six cocoyam and one taro RAPD phenotype

Group/accession	1	2	3	4	5	6	7
1. Group A	1.00						
2. Barbados	0.97	1.00					
3. Chowbutton	0.97	0.95	1.00				
4. Drearies	0.89	0.86	0.86	1.00			
5. Group B	0.92	0.90	0.90	0.87	1.00		
6. Group C	0.91	0.88	0.89	0.97	0.89	1.00	
7. Colocasia	0.11	0.15	0.11	0.11	0.11	0.10	1.00

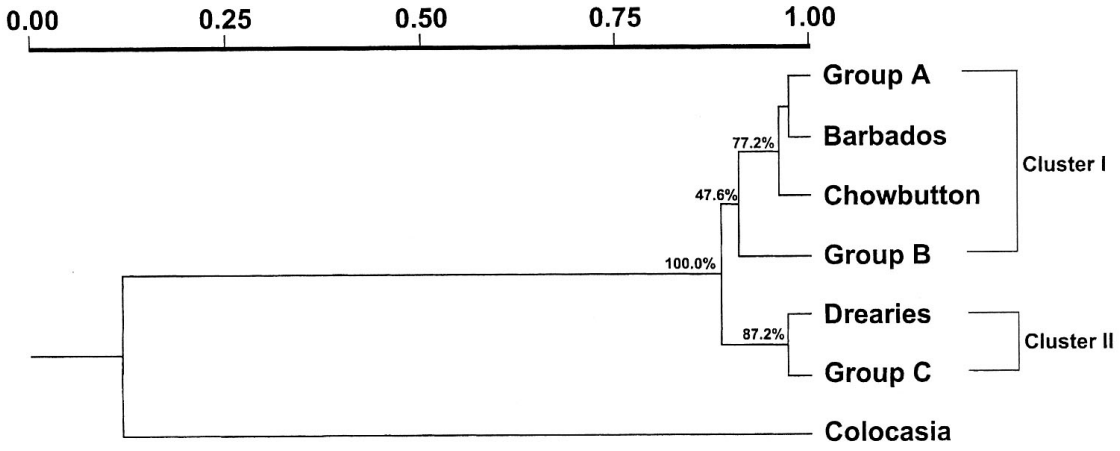

Fig. 2. UPGMA based dendrogram of cocoyam and taro accessons generated from 40 RAPD markers. The numerical scale indicates genetic similarity. Accession groups correspond to Table 1. Confidence limits for dendrogram based on 1000 bootstrap replications.

Based on their genetic distances, relationships among the six groups of cocoyams are represented in Fig. 2. Bootstrap *P* values are indicated at the corresponding node for each cluster. Two clusters were clearly resolved. Cluster I contained Group A, Barbados, Chowbutton, and Group B. Cluster II contained Drearies and Group C. Within Cluster I two sub-groups were resolved with a low *P* value (47%). This was probably caused by the inclusion of Group B, which contained the two identical accessions classified as *X. violaceum*. The node internal in the cluster had a high *P* value (77%) and reflected the high genetic similarity between Group A, Barbados, and Chowbutton. Cluster II had a high bootstrap *P* value (87%) again reflecting the high genetic similarity values between Drearies and Group C.

In many clonally propagated species mutations arise as bud sports. These are often selected and given a new name and are almost identical genetically to the mother clone. This may explain the unexpected results for group C. Santo

Domingo Purple has violet interior cormel color and is classified as *X. violaceum* while Alela has white interior cormel color and is therefore classified as *X. caracu*. *Xanthosoma* species have been differentiated using morphological traits. Expression of some traits can be greatly influenced by the environment making classification difficult. An additional source of confusion occurs when an accession is moved to another growing area and given a new name. This can been seen among the accessions in Group A where Florida White, Santo Domingo White, Blanca Espanola, Blanca del Pais and Blanca are identical for RAPD banding patterns but have different local names.

Cocoyam accessions seldom flower. The flowers are protogynous, the stigma is receptive before the pollen is shed, thus severely restricting seed set. These plants, both *X. caracu* and *X. violaceum*, can be artificially self and cross-pollinated and will produce significant numbers of seed under proper conditions (Volin and Zettler, 1976). Progeny from between species crosses are fertile suggesting that *X. caracu* and *X. violaceum* are variant forms of the same species (Goenaga and Hepperly, 1990). The amount of genetic diversity within this group of species known as cocoyam is unknown. The ability of cocoyam to reproduce sexually has been demonstrated; however, it is rare. This ability suggests that genetic diversity may be present among domesticated genotypes and wild material but not currently present within this collection.

The accessions used in this study, which represent 25% of the total accessions in the collection at TARS, are of limited value as a genetic resource. Based on our results, there is a high probability that many of the accessions in the collection not included in this study are actually duplicates with different common names. Therefore, to increase the range of genetic variation new accessions must be added. *Xanthosoma* was domesticated in tropical America and West Indies in pre-Columbian times and these areas are considered the center of diversity for the genus (Purseglove, 1972). The Antilles seem to have more indigenous varieties than any of the mainland countries of central or northern South America and should be a starting point for additional sampling. The RAPD analysis reported here confirms observations made using traditional phenotypic evaluation. It also demonstrates the usefulness of molecular data in the management of germplasm collections.

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